Remarks

In the Office Action dated June 15, 2004, claims 1-15, in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks. Claims 1-15 remain in this application and new claims 16-18 have been added to the application.

The office action indicates that the trademarks NUCLEOSIL™ AND Pico-Tag™ should be capitalized and accompanied by generic terminology. The specification has been amended as requested. Attached to this response are references which show that the generic terminology inserted into the specification does not introduce new matter.

Claims 1-15 were rejected under 35 USC §112, first paragraph as lacking enablement. Applicants respectfully point out that signal transduction by BMP proteins is effected by the binding of a BMP protein to a serine-threonine kinase receptor, of which there are two types, type I and type II. Yamashita et al. disclose that there are various type I receptors which bind BMPs, for instance, BMPR-Ia, BMPR-Ib and ActR-I, of which only the first two are BMP-specific, whereas the latter also binds activin. The various BMP proteins also vary with regard to their affinities for the different type I receptors. Page 571 of Yamashita et al. states that BMPR-11 is a BMP-specific type 11 receptor which binds several BMPs, e.g. BMP-2, BMP-4, BMP-7 (OP-1) and MP52 (GDF-5). In addition, there are type II receptors, ActR-II and ActR-IIb, which bind BMPs but also activins. BMPR-I (a and b) and BMPR-11 receptors play a critical role in

bone formation (the paragraph spanning pages 572 and 573 of Yamashita et al.). As shown in Fig. 2 of the review article by Yamashita et al. (Bone, vol.19, 199fi), these type I and type II receptors form a heterotetrameric complex which is induced by ligand binding. It is important to note that although BMPs are capable of binding separately or to either a type I or a type II receptor, both types are necessary for signal transduction. This means that, if one of the two receptor types is not available for signal transduction, there will be no signal transduction.

As a consequence, when a modified MP52 binds to the BMPR-11 receptor inhibiting signal transduction, it is immaterial whether the binding partner of the BMPR-11 receptor in said heterotetrameric complex is a BMPR-Ia or BMPR-Ib type. In both cases, the BMPR-11 receptors are occupied and are therefore no longer available for binding by other BMP proteins. This means that none of the other BMPs such as BMP-2, BMP-4 or BMP-7 are able to bind to BMPR-11 which, in turn, means that these BMPs are also incapable of inducing signal transduction. This is the reason why, for instance, a modified MP52 protein will not only inhibit signal transduction by wildtype MP52 but also by other BMP proteins.

Similarly, it is of no importance that other BMPs can bind to BMPR-Ia as can be seen from Yamashita et al., page 571. MP52 (GDF-5) binds preferably to BMPR-Ib and not BMPR-Ia. In spite of this preference, MP52 is still effective as an antagonist for BMP-2 because both bind to BMPR-11 and, therefore, there is not enough BMPR-11 available for binding of BMP-2. Since BMP-4 and BMP-7 also bind to BMPR-II, it is clear that for these BMPs there is also no BMPR-11

receptor available for binding. Thus, even if there is an excess of BMPR-Ia, there will be no signal transduction because there is no BMPR-11 available which in this case represents the limiting factor. The same is, of course, true for modified BMP-4, BMP-2 and BMP-7 which also bind to BMPR-II. In summary, the above mechanism explains why a modified BMP protein can act as an antagonist not only for wildtype BMP proteins of the same type but also other BMP family members.

In addition to the above, the individual members of the BMP subfamilies have significant structural similarities. It is assumed that the hydrophobic amino acids, which are modified in the present invention, are important for the activity (see the line spanning pages 7 and 8 of the present application: "It is assumed that these hydrophobic amino acids play an important role for genetic activity". It is thought that the hydrophobic amino acids of the dimeric BMPs form hydrophobic pockets which can interact with the hydrophobic portion of the receptor. The amino acids Trp 28/Trp 31 and Trp 28/Val 67 of the two monomers of a dimeric BMP-2 are very close together and presumably form a hydrophobic pocket which binds to BMPR-1. The other BMP proteins BMP-2, BMP-4 and BMP-7 also have hydrophobic amino acids at the corresponding positions. It is therefore reasonable to assume that BMP-3, BMP-4, BMP-7 and MP52 all form some kind of hydrophobic pocket which is indispensable for functional interaction with the receptor. Yamashita et al. also note on page 569 that "it is likely, however, that the mechanisms whereby BMPs and other members of the TGF-P family transduce signals are analogous".

In view of the above discussed underlying mechanism of signal transduction of BMPs, one skilled in the art would indeed expect a modified form of BMP-2 or BMP-4 to be an effective antagonist against other BMPs and applicants request that this rejection be withdrawn.

Claims 1-15 were rejected under 35 USC §112, first paragraph, as lacking an adequate written description. Applicants respectfully point out page 12, lines 6-13 of the present application which indicates that the examples show that a mature modified MP52 is not only active as an antagonist against MP52 but also against BMP-2. As discussed above, MP52, BMP-2, BMP-4 and BMP-7 all bind to BMPR-II and thus one it was determined that a modified MP52 inhibits signal transduction by BMP-2 proteins, it was clear that the modified MP52 and modified BMPs would have the same antagonistic activity against other BMPs since they all use the same general mechanism. Applicants contend that the present application provides an adequate written description of modified MP52 and BMP proteins and their antagonistic activity against BMP proteins and request that this rejection be withdrawn.

Claims 1, 2, 4, 5, 8 and 12-15 were rejected under 35 USC §112, first paragraph as lacking enablement for an MP52 protein modified at residue 111 alone because said residue is not located in the receptor binding site. Claim 1 has been amended to indicate that the modified residue is in the receptor biding site. In view of this amendment applicants request that this rejection be withdrawn.

Applicants respectfully submit that all of claims 1-18 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

By

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Enclosures: NUCLEOSIL and PICO.TAG catalog pages

Waters
Lab Highlights

An Internal Communication
of Applications and Techniques

LAH 0220 12/84
DP/LS/MD/AA/DV

WATERS ANNOUNCES PICO·TAG ANALYSIS

A PRECOLUMN DERIVATIZATION METHODOLOGY FOR AMINO ACID ANALYSIS

In recent years the use of reverse-phase HPLC has become increasingly widespread for the separation and quantitation of derivatized amino acids. This rising popularity is especially evident with those researchers involved with low level amino acid analysis of rare or valuable samples as well as laboratories that are overburdened with samples that conventional ion-exchange systems are too slow and too insensitive to handle.

The most prevalent reagents used for precolumn techniques have been ortho-phthalaldehyde (OPA) and dansyl or dabsyl chloride. However, OPA does not react with proline and hydroxyproline, and derivative stability is a problem, while the latter two reagents cause very large reagent peaks and can yield multiple derivatives with several amino acids, most notably histidine and tyrosine.

FIGURE 1

olumn: PICO-TAG™ Reverse-Phase
luents: A = PICO-TAG™ A
B = PICO-TAG™ B
low: 1 ml/min.

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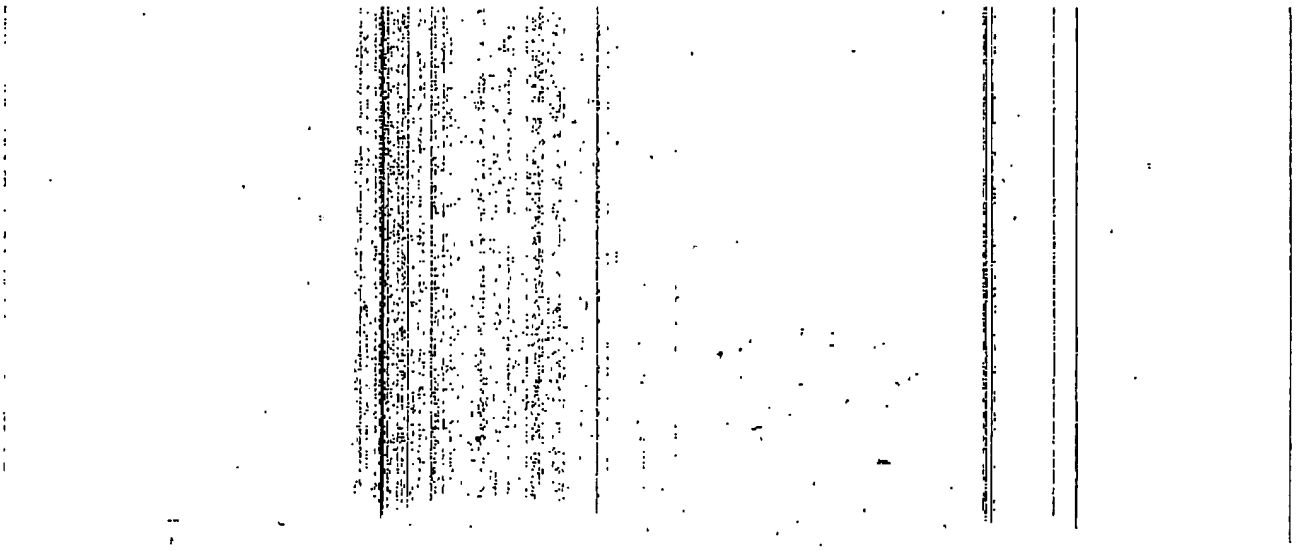

LAH 0220 12/84
DP/LS/MD/AA/DV

Now Waters introduces the PICO·TAG™ Method, a complete, packaged hodology using phenyl isothiocyanate (PITC), the well-known Edman ncing reagent. This remarkable technique solves the most pressing blems of the other precolumn methods, yet pushes the speed and sensitivity levels that will satisfy even the most demanding scientist (1,2).

Figure 1 shows the separation of 250 pmol of amino acid standard. Note excellent resolution achieved throughout even though the analysis is plete in under twelve minutes. Of course, proline (Peak 9) responds equally well as the primary amino acids since the same chromophore is produced. y high sensitivity is illustrated in the chromatogram in Figure 2 (only 1 omole of standard!).

Other outstanding features of the PICO·TAG™ Method include ntitative reaction, excellent reproducibility and linearity in the range 500 picomoles, very good derivative stability even at room temperature, and plete reagent volatility for low interference after vacuum drying. Typical O·TAG™ results are shown in Table I with a comparison to an -exchange analysis of myoglobin.

TABLE 1

COMPARISON OF PICO-TAG™

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NUCLEOSIL®*

- **Base deactivated AB, HD and PROTECT I packings**
- **Pore size selection from 50Å to 4000Å**
- **Available in popular 125x4.0 and 250x4.0mm dimensions**

Nucleosil is a high surface area spherical silica manufactured by Macherey-Nagel. This high quality silica has an extremely narrow particle size distribution making it a very efficient packing material for HPLC.

When choosing a Nucleosil column, the compound's molecular weight (MW) must first be taken into account. Most researchers choose the 100Å silicas for compounds with MW below 2000 daltons; in general, the 50 and 120Å material are suitable for small molecule analysis. For proteins and other compounds with MW greater than 2000 daltons, the 300Å and larger pore size silicas are the better choice.

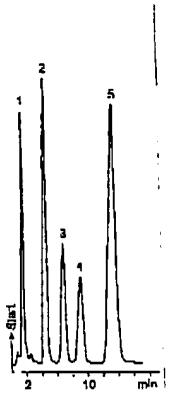
Material Characteristics

Packing Material	Particle Shape/Size (µ)	Pore Size (Å)	Pore Volume (mL/g)	Surface Area (m²/g)	Carbon Load (%)		Calculated Surface Coverage (µmoles/m²)	End Capping
Nucleosil 50 Silica	Spher. 3, 5, 7, 10	50	0.6	450	0		0	No
Nucleosil 100 Silica	Spher. 3, 5, 7, 10	100	1.0	350	0		0	No
Nucleosil 100 C18	Spher. 3, 5, 7, 10	100	1.0	350	14,	Monomeric	2.05	Yes
Nucleosil 100 C18 AB	Spher. 5	100	1.0	350	25,	Polymeric	4.51	Yes
Nucleosil 100 C18 HD	Spher. 3, 5	100	1.0	350	20,	Monomeric	—	Yes
Nucleosil 100 Protect I	Spher. 5	100	1.0	350	11,	Monomeric	—	Yes
Nucleosil 100 C8	Spher. 5, 7, 10	100	1.0	350	8,	Monomeric	2.49	No
Nucleosil 100 C8 HD	Spher. 5,	100	1.0	350	13,	Monomeric	—	Yes
Nucleosil 100 C2	Spher. 7	100	1.0	350	3.5,	Monomeric	N/A	No
Nucleosil 100 CN	Spher. 5, 10	100	1.0	350	5,	Monomeric	—	No
Nucleosil 100 NH_2	Spher. 5, 10	100	1.0	350	3.5,	Monomeric	N/A	No
Nucleosil 100 Diol	Spher. 7	100	1.0	350	5,	Monomeric	—	No
Nucleosil 100 Phenyl	Spher. 5, 7	100	1.0	350	5,	Monomeric	1.98	No
Nucleosil 100 SA	Spher. 5, 10	100	1.0	350	1 mval/g,	Monomeric	—	No
Nucleosil 100 SB	Spher. 5, 10	100	1.0	350	1 mval/g,	Monomeric	—	No
Nucleosil 120 Silica	Spher. 3, 5, 7, 10	120	0.65	200	0		0	No
Nucleosil 120 C4	Spher. 5	120	0.65	200	5,	Monomeric	N/A	No
Nucleosil 120 C8	Spher. 3, 5, 7, 10	120	0.65	200	7,	Monomeric	3.27	No
Nucleosil 120 C18	Spher. 3, 5, 7, 10	120	0.65	200	11,	Monomeric	2.69	Yes
Nucleosil 120 Phenyl	Spher. 7	120	0.65	200	6,	Monomeric	2.40	No
Nucleosil 300-C18	Spher. 5, 7, 10	300	0.8	100	6,	Monomeric	2.72	Yes
Nucleosil 300-C4	Spher. 5, 7	300	0.8	100	1,	Monomeric	1.41	Yes
Nucleosil 300-C8	Spher. 5, 7	300	0.8	100	2,	Monomeric	1.72	Yes
Nucleosil 300-Phenyl	Spher. 7	300	0.8	100	2,	Monomeric	1.56	No
Nucleosil 500-C4	Spher 7	500	0.8	35	1,	Monomeric	2.04	Yes
Nucleosil 500-C8	Spher. 7	500	0.8	35	1,	Monomeric	2.42	No
Nucleosil 500-C18	Spher. 7	500	0.8	35	2,	Monomeric	2.45	Yes
Nucleosil 1000-C4	Spher. 7	1000	0.85	25	1,	Monomeric	5.66	Yes
Nucleosil 1000-C18	Spher. 7	1000	0.85	25	1,	Monomeric	1.69	Yes
Nucleosil 4000-C4	Spher. 7	4000	0.7	10	<1,	Monomeric	N/A	Yes
Nucleosil 4000-C18	Spher. 7	4000	0.7	10	1,	Monomeric	4.22	Yes

App ID 5407

Penicillins

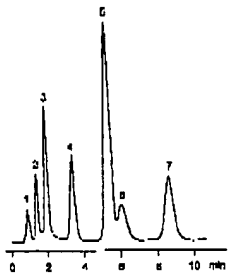
Column:	Nucleosil 5µ C18 100Å
Dimensions:	250 x 3.2mm
Order No:	00G-0323-R0
Mobile Phase:	0.05M Phosphate buffer pH 7/Acetonitrile (7:2.5, v/v)
Flow Rate:	1.6mL/min
Detection:	UV @ 220nm
Injection Volume:	10 µL
Sample:	1. Ampicillin 2. Oxacillin 3. Cloxacillin 4. Flucloxacillin 5. Dicloxacillin



App ID 5408

Narcotics

Column:	Nucleosil 10µ C18 100Å
Dimensions:	250 x 4.0mm
Order No:	00G-0330-00
Mobile Phase:	Methanol/Water (45:55)
Flow Rate:	0.42cm/sec
Detection:	UV @ 254nm
Injection Volume:	5µL
Sample:	1. Unknown 2. Veronal 3. Luminal 4. Prominal 5. Reversal 6. Unknown 7. Thiogenal



*Nucleosil® is a trademark of Macherey-Nagel

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